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SEARCH REQUEST FORM

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Title of Invention Monoclonal antibodies to Tacrolimus and immunoassay methods

Inventors (please provide full names): See page 1 attached

Earliest Priority Date: 08/03/99

Keywords (include any known synonyms registry numbers, explanation of initialisms):

Tacrolimus (structure of Fig. 16) } see page 1 attached
FR-900506
FK-506

antibod?

hybridoma

immunogen?

* Carboxymethyl oxime (see claim 64)

keyhole limpet hemocyanin

bovine serum albumin L5

Point of Contact:

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Technical Info. Specialist

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1412

1348-588

Search Topic:

Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

→ (continued)

ovalbumin L6

* bromoacetyl (see claim 76)

biotin (see claim 73-77)

glucose-6-phosphate dehydrogenase (see claim 81) L8

immunoassay

* These are preferred linkers.

Please search for TACROLIMUS used in the context of the attached claims.

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MONOCLONAL ANTIBODIES TO TACROLIMUS AND IMMUNOASSAY
METHODS FOR TACROLIMUS

by

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Kenneth C. Kasper, Henry Jeong, Dariush Davalian, Hshiou-ting Liu,
 Paul L. Miller & Denise L. Williams

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FIELD OF THE INVENTION

This invention is directed to monoclonal antibodies to tacrolimus, methods for producing such monoclonal antibodies, and derivatives of tacrolimus useful for producing such monoclonal antibodies.

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BACKGROUND OF THE INVENTION

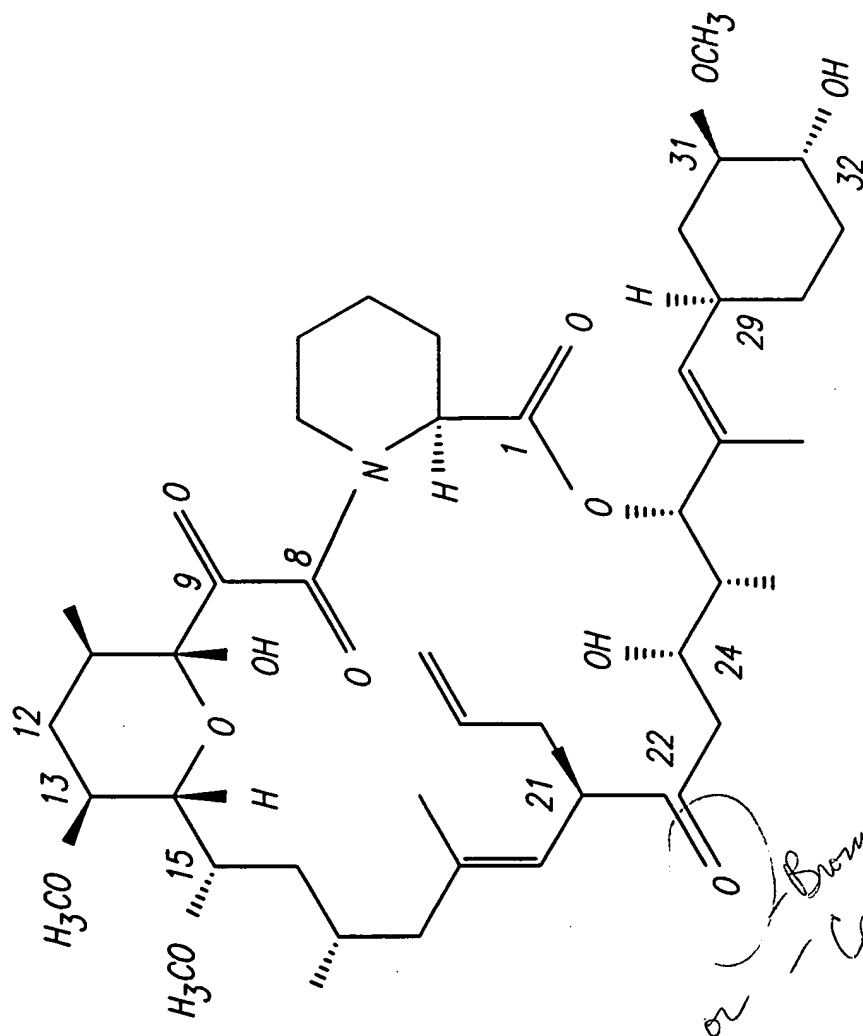
Tacrolimus is a macrolide isolated from *Streptomyces tsukubaensis*.

20 Tacrolimus has the chemical name [3S-[3R*[E(1S*, 3S*, 4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14, 16-dimethoxy-4, 10, 12, 18-tetramethyl-8-(2-propenyl)-15, 19-epoxy-3H-pyrido[2, 1-c][1, 4]-oxaazacyclotricosine-1, 7, 20, 21 (4H, 25 23H) tetrone. The structure of tacrolimus, giving the numbering, is shown below in Figure 16.

Tacrolimus is also known as FR-900506 or FK-506. Tacrolimus has immunosuppressive activity and antimicrobial activity.

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or (- Bromoacetyl here
- Carboxymethyl oxime
here

FIG. 16

We claim:

1. A monoclonal antibody to tacrolimus that is an IgG₁λ monoclonal antibody that has a binding affinity for tacrolimus of about 3.7×10^9 liters/mole, that cross-reacts with 13-demethyl tacrolimus, and that has less than about 8% cross-reactivity to all of the following tacrolimus metabolites: 15-demethyl tacrolimus; 31-demethyl tacrolimus; 13, 31-didemethyl tacrolimus; 15, 31-didemethyl tacrolimus; and 12-hydroxy tacrolimus and is designated 1H6.

2. A monoclonal antibody to tacrolimus that:

(a) competes with the IgG₁λ monoclonal antibody designated 1H6 at least about 80% as effectively on a molar basis as compared with the IgG₁λ monoclonal antibody designated 1H6 as measured by competition assays; and

(b) has less than about 10% cross-reactivity with each of 15-demethyl tacrolimus, 31-demethyl tacrolimus, 13, 31-didemethyl tacrolimus, 15, 31-didemethyl tacrolimus, and 12-hydroxy tacrolimus.

3. The monoclonal antibody of claim 2 wherein the antibody competes at least about 90% as effectively on a molar basis as the monoclonal antibody designated 1H6 and has less than about 8% cross-reactivity with each of 15-demethyl tacrolimus, 31-demethyl tacrolimus, 13, 31-didemethyl tacrolimus, 15, 31-didemethyl tacrolimus and 12-hydroxy tacrolimus.

4. A hybridoma producing an IgG₁λ monoclonal antibody to tacrolimus designated 1H6 that is an IgG₁λ monoclonal antibody that has a binding affinity for tacrolimus of about 3.7×10^9 liters/mole, that cross-reacts with 13-demethyl tacrolimus, and that has less than about 8% cross-reactivity to all of the following tacrolimus metabolites: 15-demethyl tacrolimus; 31-demethyl tacrolimus; 13, 31-didemethyl tacrolimus; 15, 31-didemethyl tacrolimus; and 12-hydroxy tacrolimus.

5. A hybridoma producing the monoclonal antibody of claim 2.

6. The monoclonal antibody of claim 2 wherein at least some of the constant
5 regions of the antibody are replaced by human constant regions so that the monoclonal antibody
is humanized.

7. The monoclonal antibody of claim 6 wherein the antibody competes at
least about 90% as effectively on a molar basis as compared with the IgG₁λ monoclonal antibody
10 designated 1H6 and wherein the antibody has less than about 8% cross-reactivity with each of
15-demethyl tacrolimus, 31-demethyl tacrolimus, 13, 31-didemethyl tacrolimus, 15, 31-
didemethyl tacrolimus, and 12-hydroxy tacrolimus.

8. A single-chain recombinant antibody (sFv) including therein the variable
15 regions of an antibody to tacrolimus that:
(a) competes with the IgG₁λ monoclonal antibody designated 1H6 at least about
80% as effectively on a molar basis as compared with the IgG₁λ monoclonal antibody designated
1H6 as measured by competition assays; and
(b) has less than about 10% cross-reactivity with each of 15-demethyl tacrolimus,
20 31-demethyl tacrolimus, 13, 31-didemethyl tacrolimus, 15, 31-didemethyl tacrolimus, and 12-
hydroxy tacrolimus.

9. The monoclonal antibody of claim 8 wherein the antibody competes at least
about 90% as effectively on a molar basis as the monoclonal antibody designated 1H6 and has
25 less than about 8% cross-reactivity with each of 15-demethyl tacrolimus, 31-demethyl
tacrolimus, 13, 31-didemethyl tacrolimus, 15, 31-didemethyl tacrolimus and 12-hydroxy
tacrolimus.

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10. A monoclonal antibody to tacrolimus produced by fusion of antibody-producing cells from an antibody-producing mammal immunized with tacrolimus derivatized with a carboxymethyl oxime moiety at a carbon atom in the non-binding domain of tacrolimus conjugated to a high molecular weight protein with a suitable fusion partner.

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11. A monoclonal antibody to tacrolimus produced by fusion of antibody-producing cells from an antibody-producing mammal immunized with tacrolimus derivatized with a carboxymethyl oxime moiety at carbon atom 22 conjugated to a high molecular weight protein with a suitable fusion partner.

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12. The monoclonal antibody of claim 11 wherein the high molecular weight protein is keyhole limpet hemocyanin.

13. An antibody to tacrolimus produced by immunization of an antibody-producing mammal with tacrolimus derivatized with a carboxymethyl oxime moiety at a carbon atom in the non-binding domain of tacrolimus conjugated to a high molecular weight protein.

14. An antibody to tacrolimus produced by immunization of an antibody-producing mammal with tacrolimus derivatized with an oxime moiety at carbon atom 22 conjugated to a high molecular weight protein.

15. The antibody of claim 14 wherein the high molecular weight protein is keyhole limpet hemocyanin.

16. A conjugate comprising the antibody of claim 1 directly or indirectly conjugated to a detectable label.

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17. The conjugate of claim 16 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

5 18. The conjugate of claim 17 wherein the label is an enzyme label.

19. A conjugate comprising the antibody of claim 2 conjugated directly or indirectly to a detectable label.

10 20. The conjugate of claim 19 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

15 21. The conjugate of claim 20 wherein the label is an enzyme label.

22. A conjugate comprising the antibody of claim 6 conjugated directly or indirectly to a detectable label.

20 23. The conjugate of claim 22 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

24. The conjugate of claim 23 wherein the label is an enzyme label.

25 25. A conjugate comprising the antibody of claim 8 conjugated directly or indirectly to a detectable label.

26. The conjugate of claim 25 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

5 27. The conjugate of claim 26 wherein the label is an enzyme label.

28. A conjugate comprising the antibody of claim 10 conjugated directly or indirectly to a detectable label.

10 29. The conjugate of claim 28 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

15 30. The conjugate of claim 29 wherein the label is an enzyme label.

31. A conjugate comprising the antibody of claim 11 conjugated directly or indirectly to a detectable label.

20 32. The conjugate of claim 31 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent label, a bioluminescent label, and a particulate label.

33. The conjugate of claim 32 wherein the detectable label is an enzyme label.

25 34. A conjugate comprising the antibody of claim 13 conjugated directly or indirectly to a detectable label.

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35. The conjugate of claim 34 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent, a bioluminescent label, and a particulate label.

5 36. The conjugate of claim 35 wherein the label is an enzyme label.

37. A conjugate comprising the antibody of claim 14 conjugated directly or indirectly to a detectable label.

10 38. The conjugate of claim 37 wherein the detectable label is selected from the group consisting of an enzyme label, a radioactive label, a fluorescent label, a chemiluminescent, a bioluminescent label, and a particulate label.

15 39. The conjugate of claim 38 wherein the label is an enzyme label.

40. A method of detecting or determining tacrolimus comprising the steps of:
(a) providing a sample suspected of containing tacrolimus;
(b) reacting the sample with:
 (i) the antibody of claim 1; and
20 (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the tacrolimus analogue is labeled with a label producing a detectable signal; and
(c) observing or measuring one of:
 (i) the signal associated with tacrolimus bound to antibody;
 (ii) the signal associated with tacrolimus unbound to antibody; or
25 (iii) the total signal present;

in order to detect or determine the presence or concentration of tacrolimus in the sample.

41. The method of claim 40 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

- 5 42. A method of detecting or determining tacrolimus comprising the steps of:
- (a) providing a sample suspected of containing tacrolimus;
 - (b) reacting the sample with:
 - (i) the antibody of claim 2; and
 - (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the
- 10 tacrolimus analogue is labeled with a label producing a detectable signal; and
- (c) observing or measuring one of:
 - (i) the signal associated with tacrolimus bound to antibody;
 - (ii) the signal associated with tacrolimus unbound to antibody; or
 - (iii) the total signal present;
- 15 in order to detect or determine the presence or concentration of tacrolimus in the sample.

43. The method of claim 42 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

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44. A method of detecting or determining tacrolimus comprising the steps of:
- (a) providing a sample suspected of containing tacrolimus;
 - (b) reacting the sample with:
 - (i) the antibody of claim 6; and
 - (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the
- 25 tacrolimus analogue is labeled with a label producing a detectable signal; and
- (c) observing or measuring one of:
 - (i) the signal associated with tacrolimus bound to antibody;
 - (ii) the signal associated with tacrolimus unbound to antibody; or

(iii) the total signal present;

in order to detect or determine the presence or concentration of tacrolimus in the sample.

5 45. The method of claim 44 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

46. A method of detecting or determining tacrolimus comprising the steps of:

(a) providing a sample suspected of containing tacrolimus;

10 (b) reacting the sample with:

(i) the antibody of claim 8; and

(ii) optionally, a tacrolimus analogue; wherein one of the antibody or the tacrolimus analogue is labeled with a label producing a detectable signal; and

(c) observing or measuring one of:

15 (i) the signal associated with tacrolimus bound to antibody;

(ii) the signal associated with tacrolimus unbound to antibody; or

(iii) the total signal present;

in order to detect or determine the presence or concentration of tacrolimus in the sample.

20 47. The method of claim 46 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

48. A method of detecting or determining tacrolimus comprising the steps of:

25 (a) providing a sample suspected of containing tacrolimus;

(b) reacting the sample with:

(i) the antibody of claim 10; and

(ii) optionally, a tacrolimus analogue; wherein one of the antibody or the tacrolimus analogue is labeled with a label producing a detectable signal; and

(c) observing or measuring one of:

- (i) the signal associated with tacrolimus bound to antibody;
- (ii) the signal associated with tacrolimus unbound to antibody; or
- (iii) the total signal present;

5 in order to detect or determine the presence or concentration of tacrolimus in the sample.

49. The method of claim 48 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

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50. A method of detecting or determining tacrolimus comprising the steps of:

- (a) providing a sample suspected of containing tacrolimus;
- (b) reacting the sample with:
 - (i) the antibody of claim 11; and
 - (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the
- tacrolimus analogue is labeled with a label producing a detectable signal; and
- (c) observing or measuring one of:
 - (i) the signal associated with tacrolimus bound to antibody;
 - (ii) the signal associated with tacrolimus unbound to antibody; or
 - (iii) the total signal present;

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in order to detect or determine the presence or concentration of tacrolimus in the sample.

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51. The method of claim 50 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to

25 detect or determine the presence or concentration of tacrolimus in the sample.

52. A method of detecting or determining tacrolimus comprising the steps of:

- (a) providing a sample suspected of containing tacrolimus;
- (b) reacting the sample with:

(i) the antibody of claim 13; and
 (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the tacrolimus analogue is labeled with a label producing a detectable signal; and

(c) observing or measuring one of:

- 5 (i) the signal associated with tacrolimus bound to antibody;
 (ii) the signal associated with tacrolimus unbound to antibody; or
 (iii) the total signal present;

in order to detect or determine the presence or concentration of tacrolimus in the sample.

10 53. The method of claim 52 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

54. A method of detecting or determining tacrolimus comprising the steps of:

- 15 (a) providing a sample suspected of containing tacrolimus;
 (b) reacting the sample with:

 (i) the antibody of claim 14; and
 (ii) optionally, a tacrolimus analogue; wherein one of the antibody or the tacrolimus analogue is labeled with a label producing a detectable signal; and

20 (c) observing or measuring one of:

- (i) the signal associated with tacrolimus bound to antibody;
 (ii) the signal associated with tacrolimus unbound to antibody; or
 (iii) the total signal present;

in order to detect or determine the presence or concentration of tacrolimus in the sample.

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55. The method of claim 54 wherein the sample is reacted with a tacrolimus analogue labeled with an enzyme label and the total signal present is observed or measured to detect or determine the presence or concentration of tacrolimus in the sample.

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56. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 1; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

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57. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 2; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

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58. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 6; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

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59. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 8; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

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60. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 10; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

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61. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 11; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

62. A test kit comprising, packaged in separate containers:

- (a) the antibody of claim 13; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

63. A test kit comprising, packaged in separate containers:

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- (a) the antibody of claim 14; and
- (b) a tacrolimus analogue labeled directly or indirectly with an enzyme label.

5 64. A derivative of tacrolimus comprising tacrolimus that is derivatized with a carboxymethyl oxime moiety at a carbon atom in the non-binding domain of tacrolimus.

65. A derivative of tacrolimus comprising tacrolimus that is derivatized with a carboxymethyl oxime moiety at carbon atom 22.

10 66. A conjugate comprising the derivative of claim 64 conjugated to a high molecular weight protein.

15 67. The conjugate of claim 66 wherein the high molecular weight protein is keyhole limpet hemocyanin.

68. A conjugate comprising the derivative of claim 65 conjugated to a high molecular weight protein.

20 69. The conjugate of claim 68 wherein the high molecular weight protein is keyhole limpet hemocyanin.

25 70. A method of derivatizing tacrolimus comprising reacting tacrolimus with carboxymethoxylamine to produce a carboxymethyl oxime derivative of tacrolimus, the carboxymethyloxime moiety being located at carbon atom 22.

71. A method of producing a conjugate of tacrolimus with a high molecular weight protein comprising:

(a) reacting tacrolimus with carboxymethoxylamine to produce a carboxymethyl oxime derivative of tacrolimus, the carboxymethyl oxime moiety being located at carbon atom 22;

(b) activating the carboxymethyl oxime to produce a reactive N-hydroxysuccinimide ester; and

(c) reacting the N-hydroxysuccinimide ester with the high molecular weight protein to produce the conjugate.

72. The method of claim 71 wherein the high molecular weight protein is keyhole limpet hemocyanin.

73. A derivative of tacrolimus comprising tacrolimus substituted with a carboxymethyl oxime moiety at carbon atom 22 linked through a linker to a biotin moiety.

74. The derivative of claim 73 wherein the linker has the structure $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-NH-CO-(CH}_2\text{)}_5\text{-NH}_2$, and wherein one amine group of the linker forms an amide bond with the carboxyl group of the carboxymethyl oxime and the other amine group of the linker forms an amide bond with the carboxyl group of the biotin.

75. A method of derivatizing tacrolimus comprising:

(a) reacting tacrolimus with carboxymethoxylamine to produce a carboxymethyl oxime derivative of tacrolimus, the carboxymethyl oxime derivative being located at position 22;

(b) activating the carboxymethyl oxime to produce a reactive N-hydroxysuccinimide ester; and

(c) reacting the N-hydroxysuccinimide ester with the carboxyl group of biotin or a biotin derivative or analogue to produce a tacrolimus derivative.

76. A derivative of tacrolimus comprising tacrolimus that is derivatized with a bromoacetyl moiety at a carbon atom in the non-binding domain of tacrolimus.

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81. The conjugate of claim 80 wherein the protein is a cysteine-containing muterin of glucose-6-phosphate dehydrogenase.

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(a) reacting tacrolimus with carboxymethoxylamine to produce a carboxymethyl oxime derivative of tacrolimus, the carboxymethyl oxime derivative being located at position 22;

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(c) reacting the N-hydroxysuccinimide ester with the trifluoroacetic acid salt of bromoacetyl ethylenediamine to produce a bromoacetyl derivative of tacrolimus.